

Making heat shot competent cells

1. Grew 5 mL culture of SH17 from single colony. 9:30 A.M.
2. Measured OD600 at by 1:10 dilution (100 microliter culture:900 microliter LB). Control = LB
3. Left culture and CaCl₂ solution on ice for 15 minutes.
4. Aliquoted 1 mL culture/centrifuge tube and spun for 30 s at max speed.
5. Resuspended in 50 microliters CaCl₂ solution.
6. In one tube, added 2 microliters of pSH033 (only contains GFP + some conjugation factors). Flicked to mix and left on ice for 10 minutes.
 - a. Left other 3 tubes on ice for 30 minutes. Put CaCl₂ + 10% glycerol solution on ice.
7. In the tube with pSH033, incubated at 42 C for 1 min and put on ice for 2 min. Placed cells in 1 mL SOC and incubated for 1 h at 37C, 250 rpm.
8. After 30 minutes, the other three tubes were centrifuged for 30 s at max speed and resuspended in 50 microliters CaCl₂ + 10% glycerol solution.
 - a. Two tubes were put in -65 C in Shelly's Competent Cell box.
 - b. Added 2 microliters of pAM120 (plasmid with GFP and all conjugation factors). Flicked to mix and left on ice for 10 minutes. Incubated at 42 C for 1 min and put on ice for 2 min. Placed cells in 1 mL SOC and incubated at 1 h at 37 C, 250 rpm.
9. Plated 100 microliters and the rest of S17 cells with pSH033 on LB/Chl(17).
10. Plated all of S17 with pAM120 on LB/Tet(10).
11. Incubated plates 16 h at 37 C. Retrieve at 9:40 A.M. tomorrow.